The Pharmaceutical and Chemical Journal, 2021, 8(3):26-42

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Pulmonary Impact of Propylene Glycol Based Fog on Wistar Rats

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Abstract The increasing use of propylene glycol based theatrical fog at events in Nigeria has necessitated an evaluation of its potential toxicity. Propylene glycol based fog finds application in the professional entertainment industry, fire service, armed forces and in churches for different purposes. Therefore, this inhalational toxicological study investigated the potential health effects of theatrical fog on wistar rats. A total of (90) female wistar rats were used for the study; 30 rats each for acute (14 days), sub chronic (3 months) and chronic (6 months) studies respectively. Each set of 30 wistar rats was separated into 6 groups. Groups (2 to 5) were exposed by whole body in a chamber to propylene glycol based fog at the concentrations (15, 30, 60, 120 & 240 g/m^3), 2 hours daily, 3 days per week, for sub chronic and chronic studies respectively while group 1 which was not treated served as control. In the acute study, exposure was done 2 hours daily for 14 days. The animals were sacrificed under chloroform anesthesia at the end of each treatment's duration. The lungs were harvested, fixed and appropriately processed for histological examination. The photomicrographs obtained from the lung tissues of animals in the acute, sub chronic and chronic exposure levels all showed apparent alterations. The common observable features of the modification include; thickened inter-alveolar septum, shrunken alveolar sacs, consolidated interstitial tissue, plugged bronchiole and a region of fibrotic tissue. The result shows that propylene glycol fog is deleterious to the lungs.

Keywords Special-effect, hygroscopic, humectants, propylene glycol, fog

Introduction

The application of theatrical fog for special effect production was previously commonly observed in the professional entertainment industries such as motion picture, television productions, live-theatre concert, Night - club and amusement arena. However, following the recent manufacturing and supplying of affordable artificial fog machines, people have commenced creating special effect during ceremonies including church services, Christmas, funerals, weddings, birthdays and similar events. Nevertheless, there is a case of an 18 year old boy who was diagnosed of ethylene glycol toxicity with calcium oxalate nephropathy following inadvertent ingestion of ethylene glycol fog fluid [1]. More so, high incidence of pulmonary symptoms; cough, phlegm, wheezing, chest-tightness, shortness of breath, asthma and reduction in lung function have been reported among entertainment employees [2]. According to Robertson *et al* [3], thirteen out of twenty-nine rhesus-monkeys expired subsequent to a thirteen month exposure to propylene glycol vapor. In the same vein, the advent of E-cigarette has introduced additional conduit of exposure to propylene glycol based fog [4]. It is actually true that the theaters are beautified by special effect but the safety of propylene glycol theatrical fog and the thermal decomposition products of propylene glycol; acrolein, acetaldehyde and formaldehyde [2] are yet to be ascertained beyond doubt.

Materials and Methods

Materials

Experimental Animals

Sixty (60) 3 week old Wistar rats weighing (34-36g) were obtained from the Department of Pharmacology Animal House, University of Port Harcourt. Another 30 adult wistar rats weighing between (180 to 200g) were also acquired from same source. The rats were acclimatized to the research environment for one week before the commencement of the study. The site temperature range was (20 to 25 ºC) with relative humidity of (40 to 70 %) and 12hr light – 12hr dark sequence. Water and feed were provided ad libitum.

Test substance

The food grade propylene glycol used for fog-fluid formation for artificial-fog production for this research was obtained from Epoxy Oil serv. Nigeria Limited, in Rivers state, Nigeria.

Fog-machine

The fog machine, Fog God, or Fogger – 1500, was acquired from Emmapee International, A subsidiary of De Absolute Sound Co. Ltd, a Nigerian distributor in Rivers State.

The fog – machine has a fog flow rate specification of (4000 cubic foot /min), heater 1500w, power input AV 220-240. It is operated manually or with a remote control.

Exposing Chamber

The exposing chamber with the volume (37.5 m^3) was carefully constructed with thick plywood to guard against sudden temperature and humidity rise during the experiment.

Methods

Fog concentration determination

The fog concentrations employed in this research were calculated with the formula;

$$\textbf{Fog concentration} = \frac{\textbf{X mg / m}^{\textbf{3}}\textbf{ / sec.}\times\ \textbf{T}}{Vm^3}$$

Where **X mg / m^3 / sec** is the machine's fog flow rate, **T** is the flow time while **Vm^3** is the volume of the exposing chamber.

The fog machine used for this study has a fog flow rate specification of (4000 grain/cubic foot/minute) which is equivalent to {9153407.6422629 mg/m^3} [5].

After acclimatization, the 60 rats were randomly divided into two sections with 30 animals each for sub chronic and chronic studies. The 30 adult wistar rats formed a section for acute study.

Acute study

In this section 30 rats in six groups (A1, A2, A3, A4, A5, & A6) were used. While groups (A2, A3, A4 , A5 & A6) were exposed to theatrical fog at the concentrations (15, 30, 60,120 & 240 g/m^3) 2 hours daily for 14 days, group A1 which was not exposed to the fog served as control.

Sub-chronic study

This section of the study required 30 rats which were divided into 6 groups (S1, S2, S3, S4, S5 & S6) with 5 rats each. The five groups of rats in this section (S2, S3, S4, S5 & S6) were exposed to theatrical fog at the concentration range of (15, 30, 60, 120 & 240g/m^3) 2 hours daily, 3 days per week for 3 months. Group S1 was not treated with the fog and played the role of positive control.

Chronic Study

In this segment, while 5 groups, (C2 to C6) with 5 rats each were exposed to (15, 30, 60, 120 & 240g/m^3) 2 hours daily, 3 times per week for 6 months, group (C1) was not treated and played the role of a control.

Exposure Technique

This study adopted the Organization for Economic cooperation and development [6] protocol for Inhalation Toxicity study with modifications.

Methods of data collection

At the end of 14 days for acute, 3 months for sub-chronic and 6 month for chronic studies, both the animals treated and the controls were sacrificed under chloroform anesthesia and the lungs were harvested and fixed for histopathological assessment.

Ethical approval

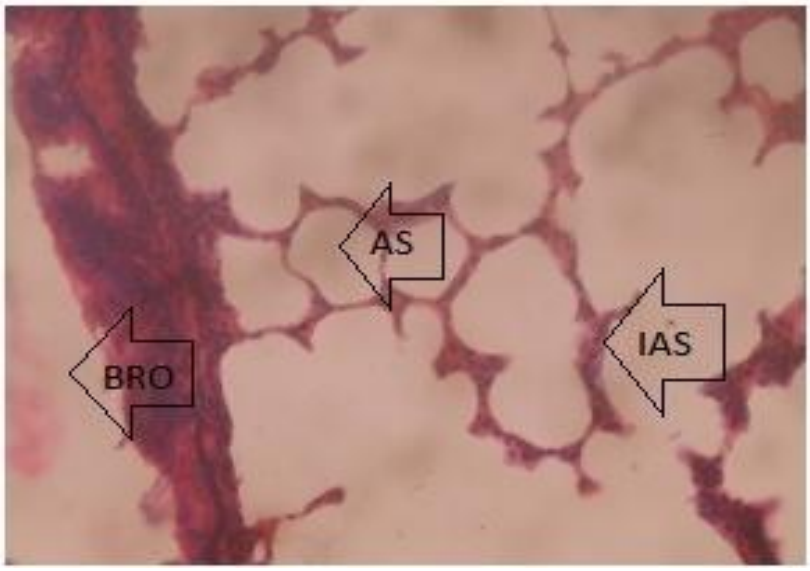
This research was endorsed by the University of Port Harcourt research ethics committee.

Results and Discussion

All lung tissues were appropriately processed, stained with haematoxylin and eosin (H & E) and viewed with a microscope at 400 magnification (Mag. X 400). The outcomes of acute, sub chronic and chronic exposures of wistar rats to propylene glycol based fog on the lungs are shown below;

Acute Effect of propylene glycol fog on Lung Histology

In the Photomicrographs of the lung tissue below; AS, IAS, BRO, BV and CON represent alveolar sac, inter-alveolar septum, bronchiole, blood-vessels and consolidation respectively.



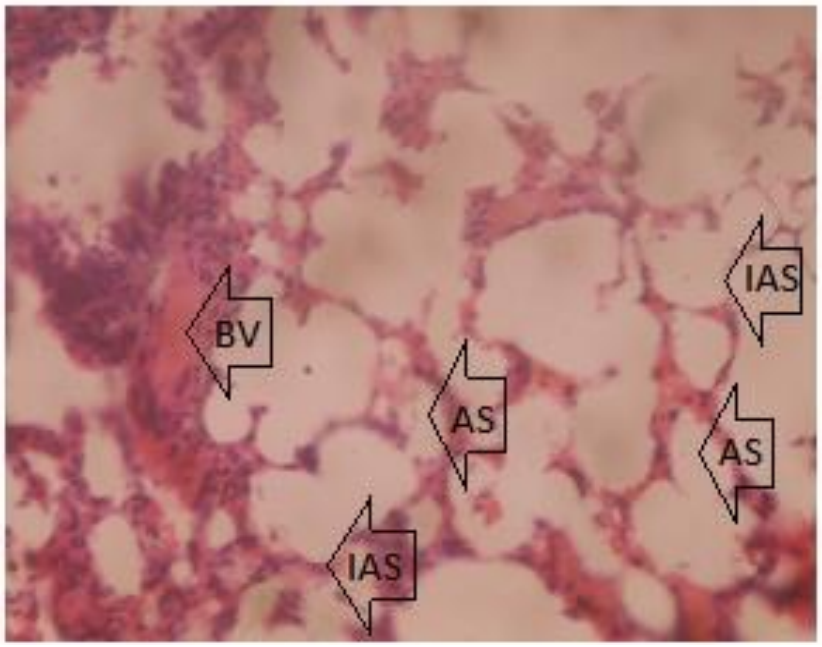
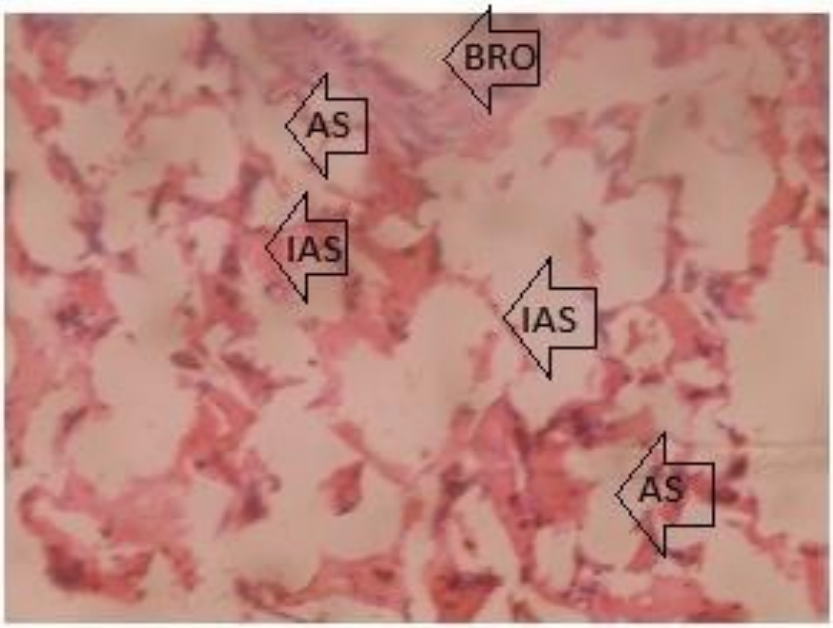


Plate 1(A & B): Photomicrographs for control showing histologically normal; AS, IAS, BV and BRO



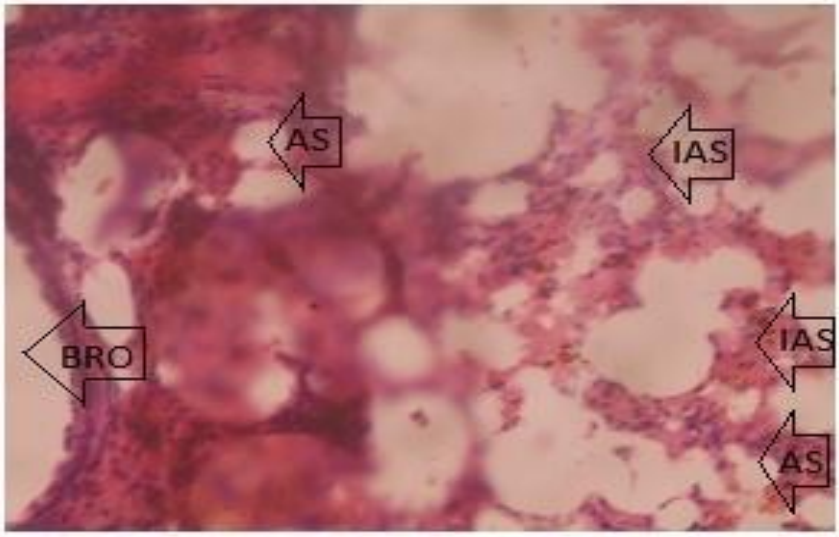
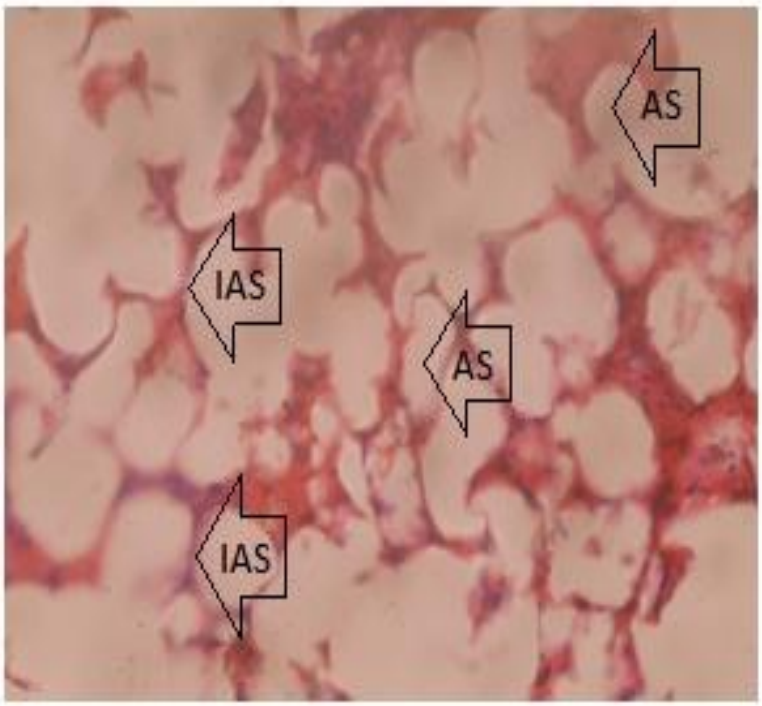


Plate 2 (A & B): Photomicrographs of the lung tissue of rats exposed to 15g/m^3 propylene glycol fog 2 hrs daily for 14 days displaying histologically normal lung features.



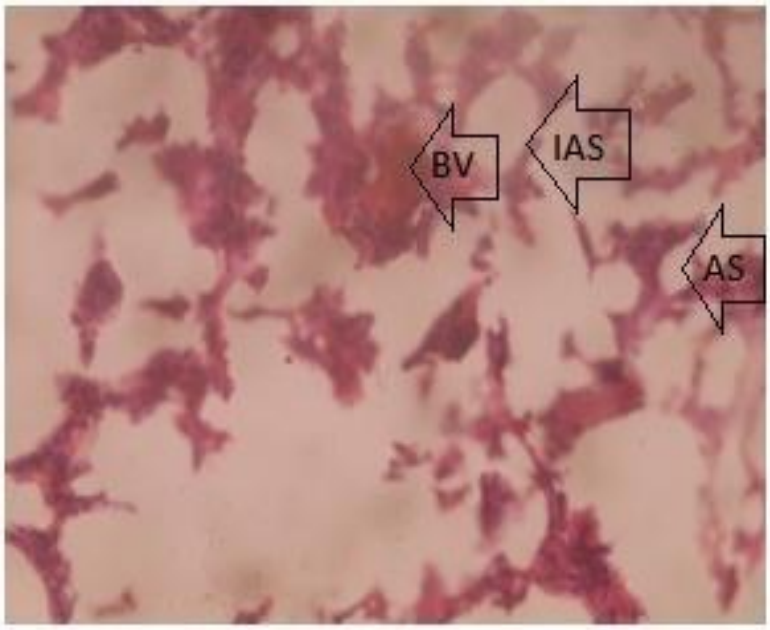
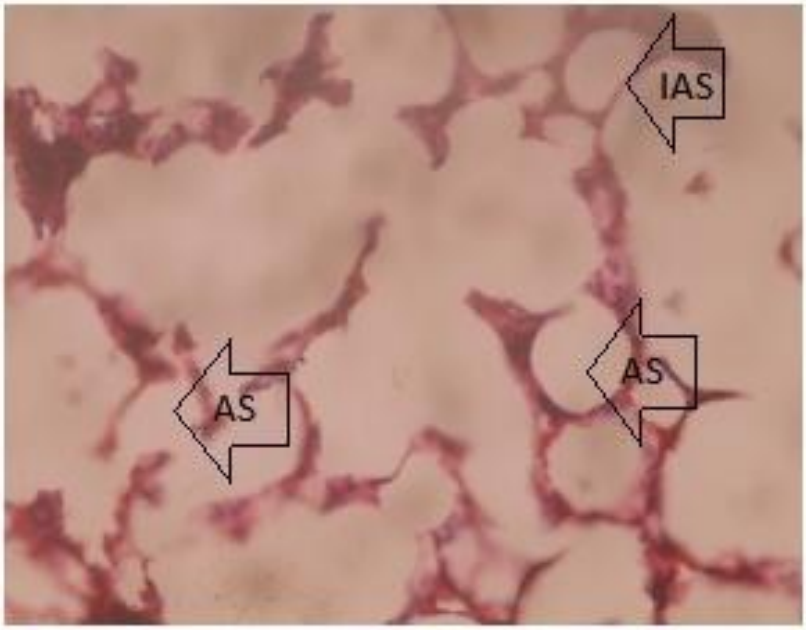


Plate 3(A & B): Micrographs of the lung tissue of rats treated with 30 g/m^3 pg fog for 14 days displaying histologically normal lung architecture



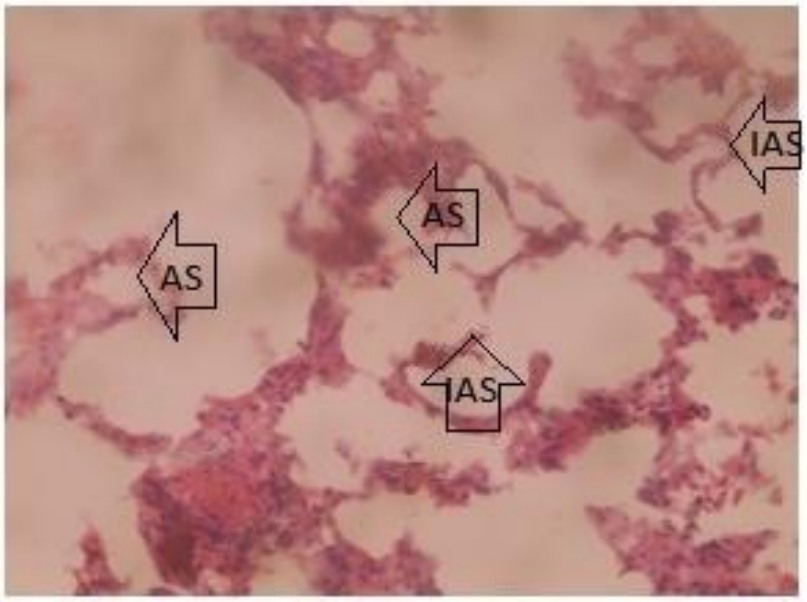
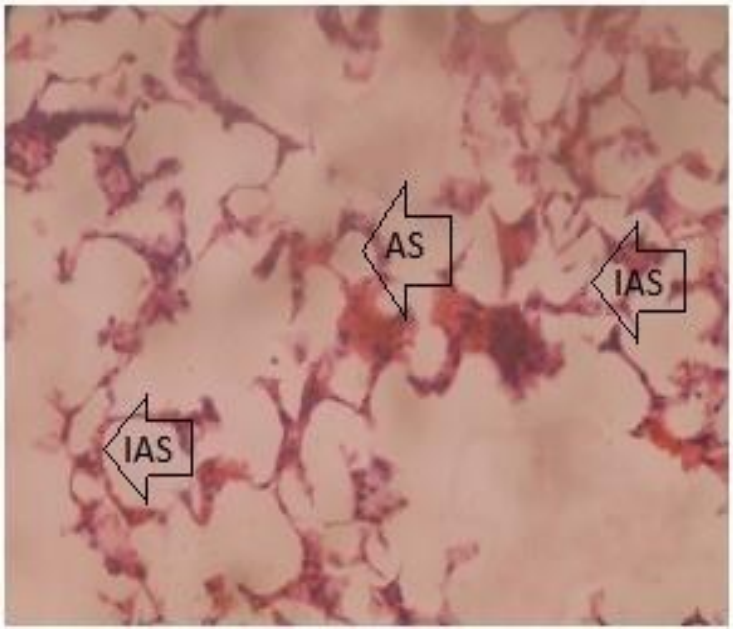


Plate 4 (A & B): Photo-micrographs of the lung tissue of rats placed on 60g/m^3 pg fog for 14 days depicting the characteristic of a histologically normal lung



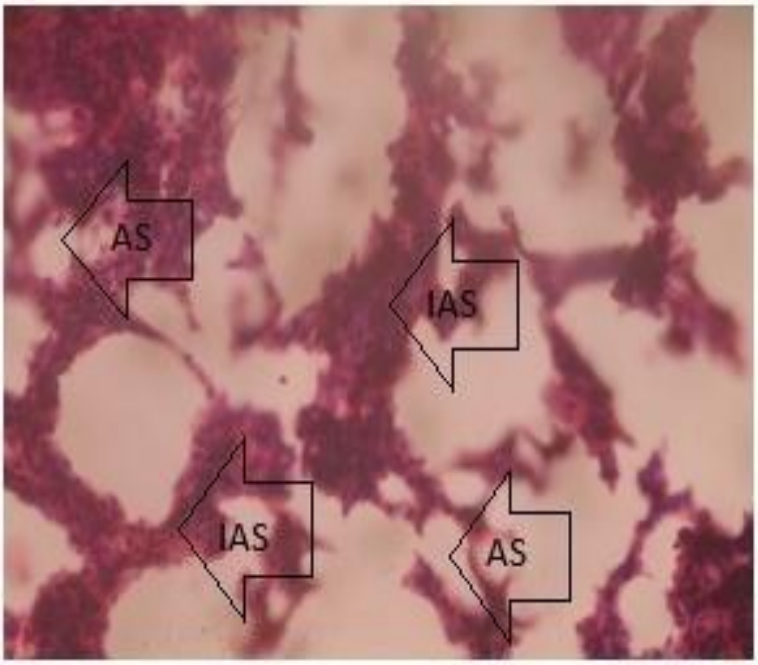
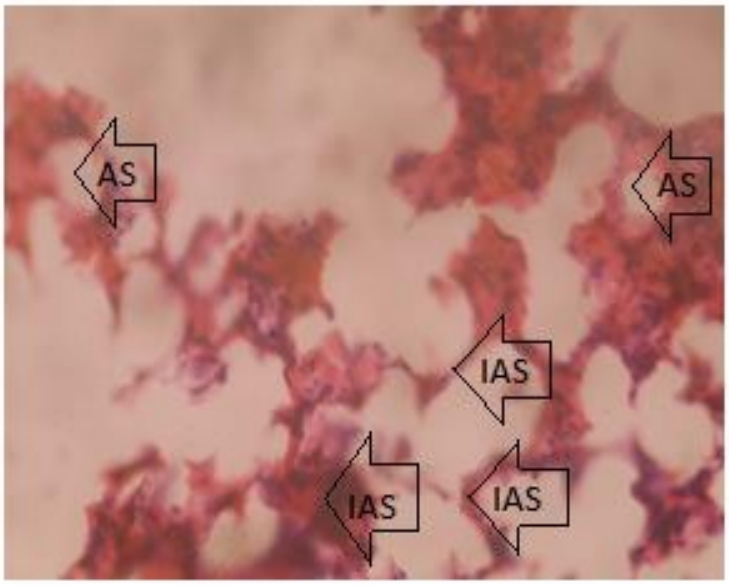


Plate 5 (A & B): Photomicrographs of the lung tissue of rats exposed to 120g/m^3 pg fog for 14 days displaying histologically distorted lung with thickened AIS and shrunken AS (B)



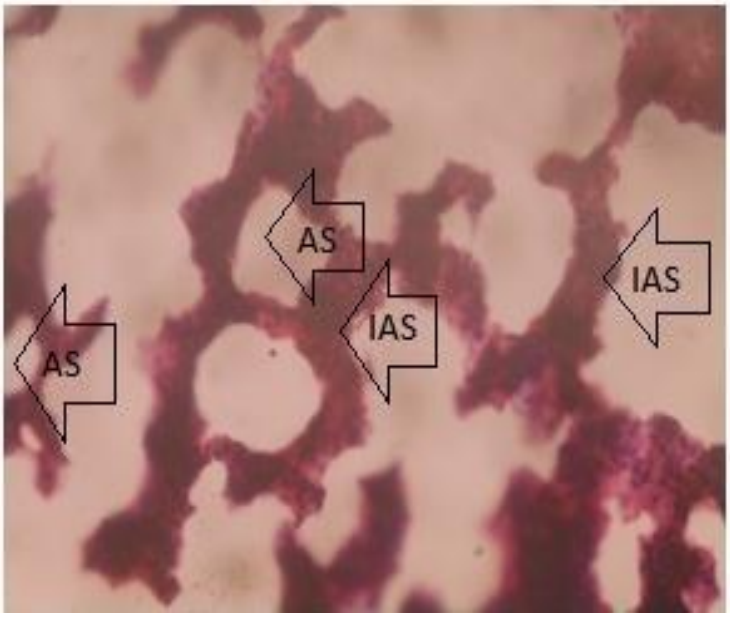
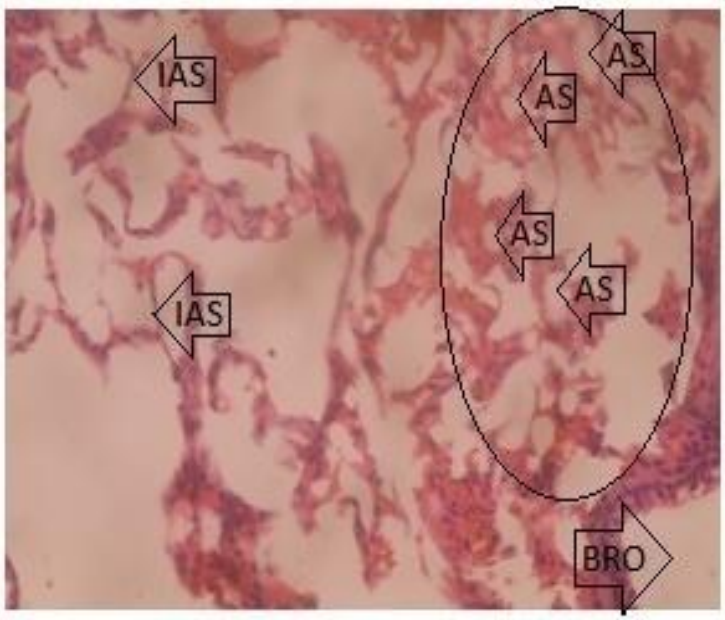


Plate 6 (A&B): Micrographs of the lung tissue of rats placed on 240g/m^3 pg fog for 14 days displaying histological distorted lung with patchy regions with thickened IAS and shrunken AS and patchy areas of thin IAS and normal AS (A) and generalized thickened IAS & normal AS (B)



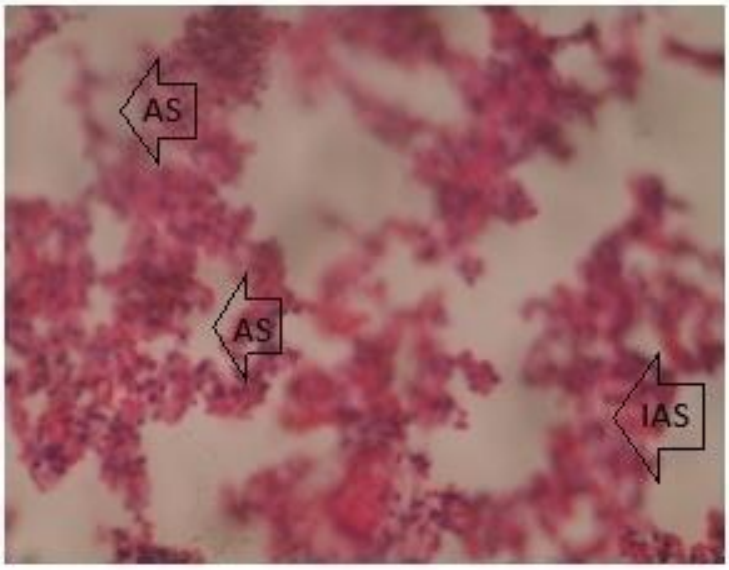
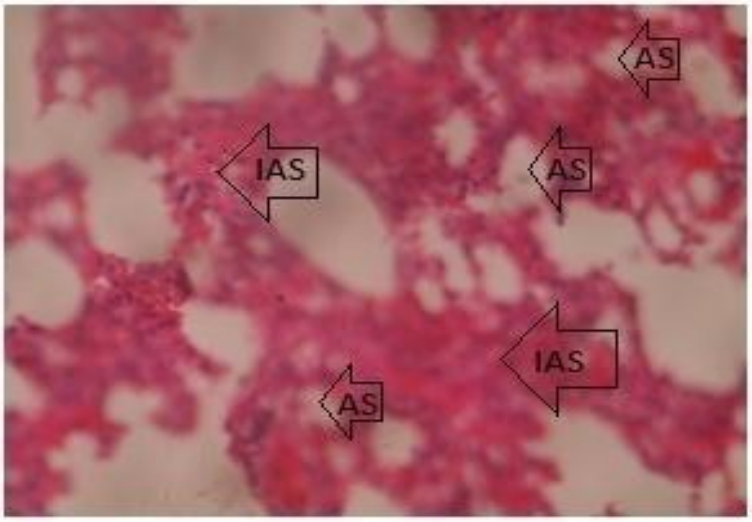


Plate 7 (A & B): Photomicrographs of the lung tissue of rats exposed to 60g/m^3 pg fog for 3 months displaying histologically distorted lung with localized (encircled) area with shrunken alveolar sacs (AS), thin IAS & patent (BRO) (A) and histological distorted lung with collapsed AS, shrunken AS & thickened IAS (B)



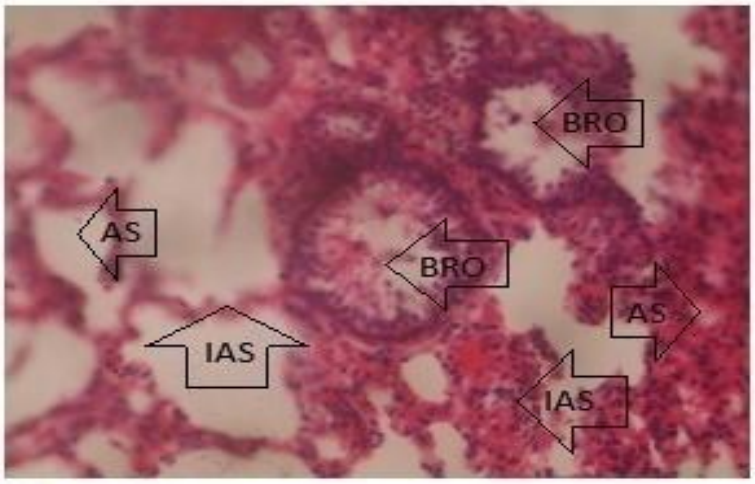
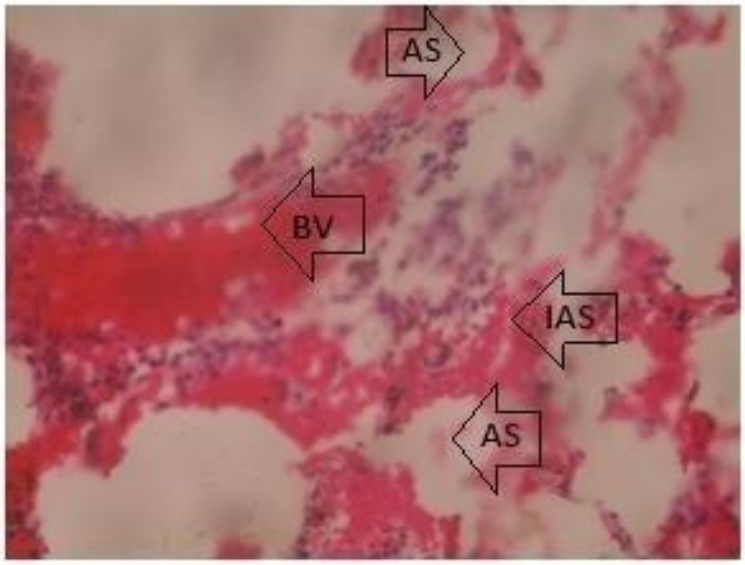


Plate 8 (A and B): Photomicrographs of the lung tissue of rats exposed to 120g/m^3 propylene based theatrical fog for 3 month showing distorted lung with shrunken AS & thickened IAS (A) and distorted lung with localized thickened IAS & shrunken AS and mucus plugged BRO (B)



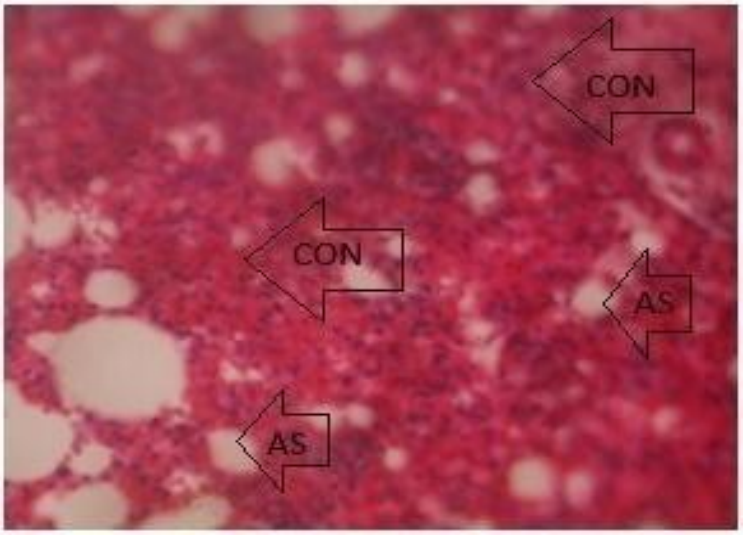
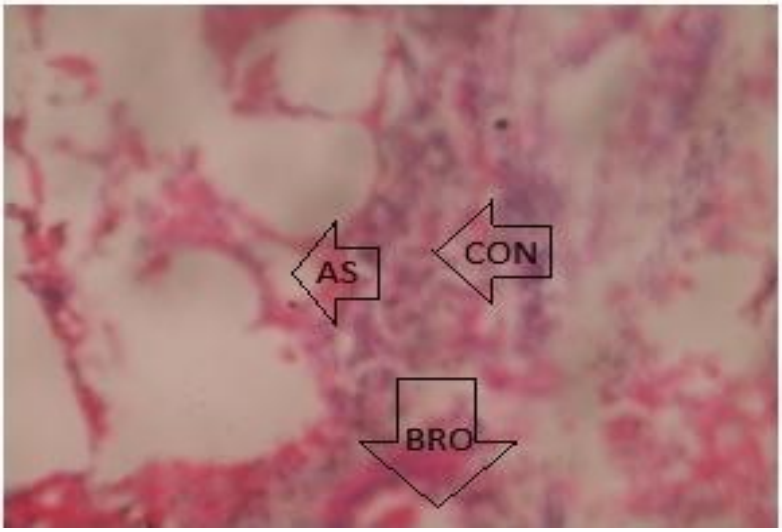


Plate 9 (A & B): Micro-graphs of the lung tissue of rats exposed to 240g/m^3 propylene based theatrical fog for 3 month depicting a distorted lung with thickened IAS & shrunken AS (A) and distorted lung with thickened IAS , shrunken AS and consolidated interstitial tissue (B)



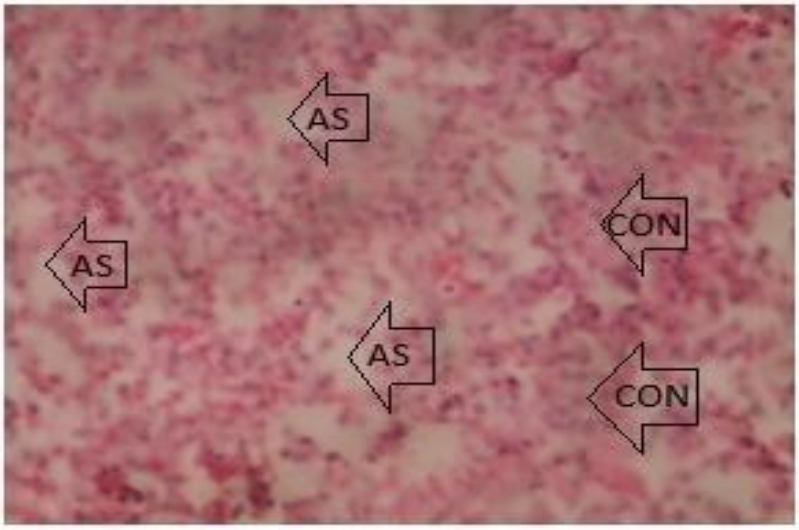
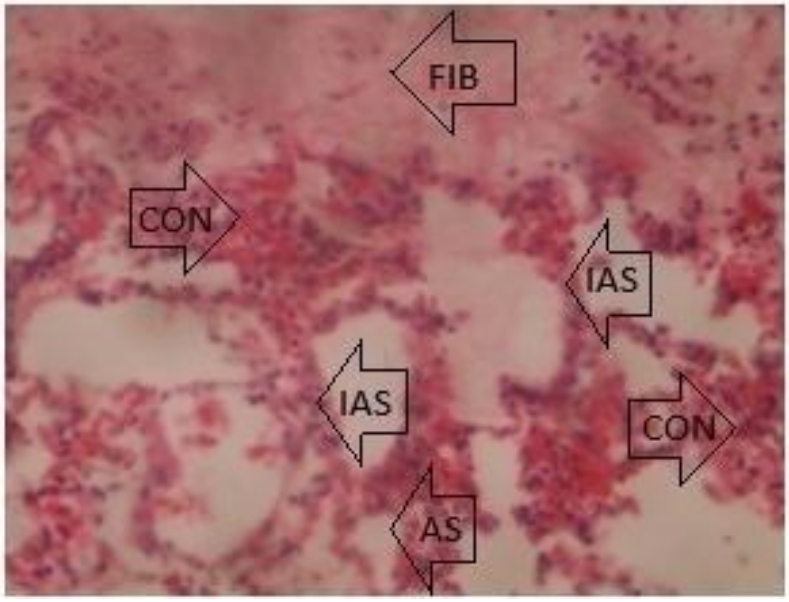


Plate 10 (A/B): Photomicrographs of the lung tissue of rats introduced to 60g/m^3 pg fog for 6 months depicting a distorted lung with shrunken AS and CON interstitial tissue (A) and a distorted lung with shrunken AS and consolidated interstitial tissue (B)



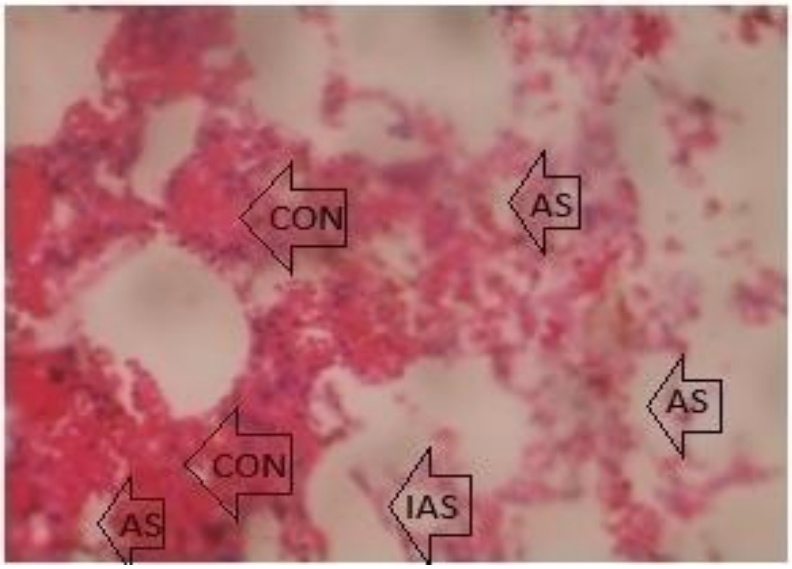
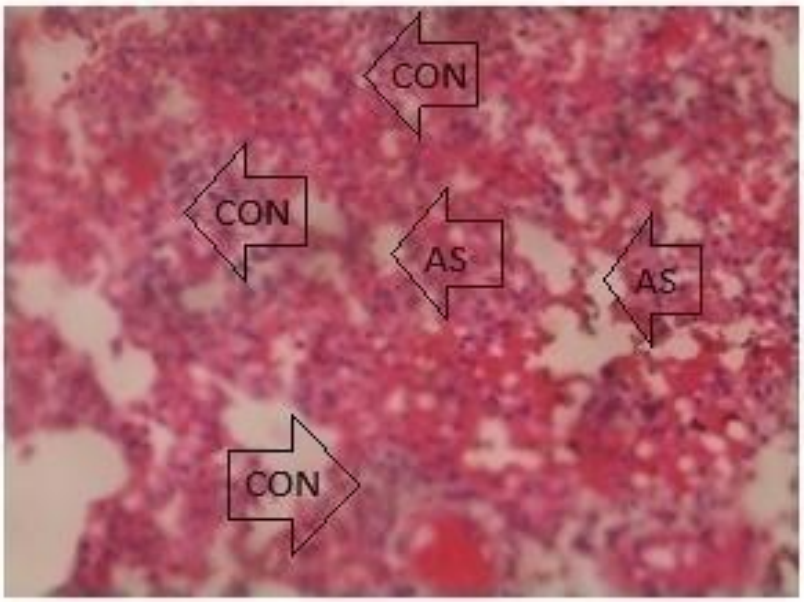


Plate 11(A/B): Photomicrographs of the lung tissue of rats exposed to 120g/m^3 propylene based theatrical fog for 6 months portraying a histologically distorted lung with thickened IAS, CON interstitial tissue, shrunken AS & Fibrotic tissue (A) and a distorted lung with shrunken AS and CON interstitial tissue (B)



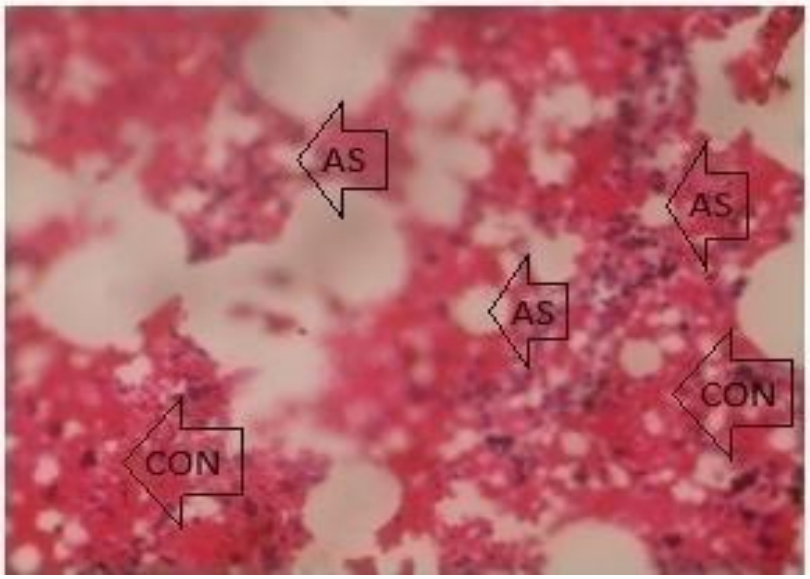


Plate12(A/B): Photo-micro-graphs of the lung tissue of rats exposed to 240g/m^3 propylene glycol fog 2 hrs daily 3 days per week for 6 months portraying a histologically distorted lung with CON interstitial tissue & shrunken AS (A) and a distorted lung with shrunken AS & CON interstitial tissue (B)

Discussion of findings

This inhalational toxicity research was done to throw more light on the potential health effect of propylene glycol based fog. The result from acute exposure demonstrates distortions in the histology of lung tissue at higher concentrations (120 & 240g/m^3) as shown on plates (5 & 6). The modifications appear more prevalent and severe among the photomicrographs obtained from the sub chronic and chronic studies. The features of the apparent alteration in the lung histology observed in this study are; thickened inter- alveolar septum, shrunken alveolar sacs, consolidated interstitial tissues, plugged bronchiole and regions with fibrotic lung tissue. According to Kay *et al* [2], a toxic substance, acrolein, is one of the thermal decomposition products of propylene glycol. Therefore the modifications in the lungs histology recorded in this investigation may be ascribed to the accumulated effect of acrolein on the lungs. Some of the several studies which have reported the injurious activity of acrolein on the lungs include; Crane *et al* [7] who reported the mortality of Sprague-Dawley rats from respiratory distress following acute exposure to acrolein. More so, Kilburn and McKenzie [8] exposed hamsters to acrolein, the results demonstrated more than 50% exfoliation of the bronchial ciliated cells, pallor and bloated conditions (48 hrs) following the exposure. Again, Ballantyne *et al* [9] treated Sprague-Dawley rats with acrolein. Symptoms for respiratory distress and hypo-activity were recorded for post-exposure days. The necropsy of the expired animals showed mottled discoloration of the lungs and clear fluid in the trachea and thoracic cavity. A histological assessment of the lungs revealed obstruction and intra-alveolar hemorrhage, fibrin deposited in the smaller airways, necrosis and exfoliated bronchiolar epithelium. Mortality was credited to lung injury. In the same vein, Cassee et al., [10-11] reported disarrangement, necrosis, thickening, desquamation, and basal cell hyperplasia in the nasal respiratory and transitional epithelium, subsequent to a 6 hour daily for 3 days nose only exposure of rats to acrolein vapor. Furthermore, the frequent complaints of respiratory symptoms; cough, phlegm, wheezing, tightness of chest, shortness of breath, asthma and reduced lung function by entertainment workers, reported by Kay *et al* [2], appears factual compared with the current finding. The mechanism of propylene glycol based fog deleterious upshot on the lungs is not clearly understood. However, the suggested mechanisms include acrolein activation of macrophages and corresponding elevations in ROS and expression of pro-inflammatory cytokines, culminating in inflammation and cells destruction, Yang et al., [12]. Based on this suggested mechanism, the thickened inter-alveolar septum, shrunken alveolar sacs, plugged bronchiole and consolidated interstitial tissues observed in this study may be the

Consequences of inflammation condition. It should also be noted that propylene glycol fog can induce Lung injury through the hygroscopic property of its fundamental component propylene glycol. It absorbs moisture from its vicinity, therefore, when propylene glycol contacts the mucus membrane it dehydrates it, causing the tissues to dry up creating tissue splits and bleeding [13].

Summary of findings

After inhalation exposure of wistar rats to propylene glycol based fog at the concentration range of (15, 30, 60, 120 & 240g/m^3) at acute, sub chronic and chronic intervals, the photomicrographs of the lung tissues obtained from the three study intervals demonstrated a time related and concentration independent modifications on the histology of lungs tissue. The alterations appeared commonly in the form of; thickened inter-alveolar septum, shrunken alveolar sacs, consolidated interstitial tissues, plugged bronchiole and regions of fibrotic tissue.

Conclusion

In conclusion, the findings from this inhalation toxicity study have provided a broad toxicity profile of propylene glycol based fog. Propylene glycol related fog is deleterious to the lungs evidenced by induction of alterations in the normal lungs histological architecture.

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